Second Edition

RNeasy® Mini Handbook

RNeasy Mini Kit

RNeasy Plant Mini Kit

For total RNA minipreps from

- animal cells
- animal tissues
- bacteria
- yeast
- plants
- filamentous fungi

For RNA cleanup

May 1999

QIAGEN

Trademarks

Patented or patent-pending and/or registered or registration-pending trademarks of QIAGEN: QIAGEN®, QIAamp®, RNeasy®, Oligotex™, and QIAshredder™.

DU is a registered trademark of Beckmann Instruments Inc.
Ultra Turrax is a registered trademark of IKA-Analysentechnik GmbH.
Polytron is a registered trademark of Kinematica.
Corex is a registered trademark of Corning Glass Works.
RNasin is a registered trademark of Promega Corp.
Nonidet is a registered trademark of Shell Chemicals.
Tissuemizer is a registered trademark of Tekmar, Inc.
Tissue-Tearor is a trademark of Bio-Spec Products, Inc.

The PCR process is covered by US Patents 4,683,195 and 4,683,202 and foreign equivalents issued to Hoffmann-La Roche AG.

Germany	**QIAGEN GmbH**	Max-Volmer-Straße 4 • 40724 Hilden Orders 02103-892-230 • Fax 02103-892-233 • Technical 02103-892-240
USA	**QIAGEN Inc.**	28159 Avenue Stanford • Valencia • CA 91355 Orders 800-426-8157 • Fax 800-718-2056 • Technical 800-DNA-PREP (800-362-7737)
Australia	**QIAGEN Pty Ltd** ACN 072 382 944	PO Box 25 • Clifton Hill • Victoria 3068 Orders 03-9489-3666 • Fax 03-9489-3888 • Technical 03-9489-3666
Canada	**QIAGEN Inc.**	2900 Argentia Road • Unit 23 • Mississauga • Ontario • L5N 7X9 Orders 800-572-9613 • Fax 800-713-5951 • Technical 800-DNA-PREP (800-362-7737)
France	**QIAGEN S. A.**	3 avenue du Canada • LP 809 • 91974 Courtaboeuf Cedex Orders 01-60-920-920 • Fax 01-60-920-925 • Technical 01-60-920-930
Japan	**QIAGEN K.K.**	Hakusan Takayanagi Bldg. 8F • 7-6, Hakusan 1 Chome • Bunkyo-ku, Tokyo 113-0001 Telephone 03-5805-7261 • Fax 03-5805-7263 • Technical 03-5805-7261
Switzerland	**QIAGEN AG**	Auf dem Wolf 39 • 4052 Basel Orders 061-319-30-30 • Fax 061-319-30-33 • Technical 061-319-30-31
UK	**QIAGEN Ltd.**	Boundary Court • Gatwick Road • Crawley • West Sussex, RH10 2AX Orders 01293-422-911 • Fax 01293-422-922 • Technical 01293-422-999

www.qiagen.com

Contents

Kit Contents

RNeasy Mini Kits* Catalog No.	(20) 74103	(50) 74104	(250) 74106
Preparations per Kit	20	50	250
RNeasy Mini Spin Columns (pink)	20	50	250
Collection Tubes (1.5-ml)	20	50	250
Collection Tubes (2-ml)	20	50	250
Buffer RLT†	18 ml	45 ml	220 ml
Buffer RW1†	18 ml	45 ml	220 ml
Buffer RPE‡	5 ml	11 ml	55 ml
RNase-free Water	10 ml	10 ml	50 ml
Handbook	1	1	1

RNeasy Plant Mini Kits* Catalog No.	(20) 74903	(50) 74904
Preparations per Kit	20	50
RNeasy Mini Spin Columns (pink)	20	50
QIAshredder Spin Columns (lilac)	20	50
Collection Tubes (1.5-ml)	20	50
Collection Tubes (2-ml)	20	50
Buffer RLT†	18 ml	45 ml
Buffer RLC†	18 ml	45 ml
Buffer RW1†	18 ml	45 ml
Buffer RPE‡	5 ml	11 ml
RNase-free Water	10 ml	10 ml
Handbook	1	1

* *Buffers available separately as RNeasy Buffer Set. Buffer RLT available separately. See ordering information (page 61).*

† *Not compatible with disinfectiing reagents containing bleach. Contains a guanidine salt which is an irritant. Take appropriate safety measures.*

‡ *Buffer RPE is supplied as a concentrate. Before using for the first time add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.*

Storage Conditions

RNeasy® Kits should be stored dry at room temperature (15 to 25°C) and are stable for at least 9 months under these conditions.

Product Use Limitations

RNeasy Kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN® product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (listed on the last page).

Technical Assistance Click Here for Contact Addresses

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding RNeasy or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors listed on the last page.

Introduction

RNeasy Mini Kits and RNeasy Plant Mini Kits are designed to isolate total RNA from small quantities of starting material. They provide a fast and simple method for the preparation of up to 100 µg of total RNA from animal cells and tissues, bacteria, and yeast (**RNeasy Mini Kits**) or plant cells and tissues and filamentous fungi (**RNeasy Plant Mini Kits**). In addition, all RNeasy Kits can be used to desalt or to purify RNA from enzymatic reactions such as DNase digestion, proteinase digestion, RNA ligation, and labeling reactions.

RNeasy Mini Kits and RNeasy Plant Mini Kits make multiple, simultaneous processing of a wide variety of biological samples possible in less than 30 min. Time-consuming and tedious methods, such as CsCl step-gradient ultracentrifugation and alcohol precipitation steps, or methods involving the use of toxic substances such as phenol and/or chloroform, are replaced by the RNeasy procedure. The purified RNA is ready for use in standard downstream applications such as:

- RT-PCR
- Poly A^+ RNA selection
- RNase/S1 nuclease protection
- Differential display
- Northern, dot, and slot blotting
- Primer extension
- cDNA synthesis

A list of references describing the use of RNeasy Kits in a variety of applications can be found on page 52.

The RNeasy Principle and Procedure

The RNeasy procedure represents a novel technology for RNA isolation. This technology combines the selective binding properties of a silica-gel-based membrane with the speed of microspin technology. A specialized high-salt buffer system allows up to 100 µg of RNA longer than 200 bases to bind to the RNeasy silica-gel membrane. Biological samples are first lysed and homogenized in the presence of a highly denaturing guanidine isothiocyanate (GITC)-containing buffer which immediately inactivates RNases to ensure isolation of intact RNA. Ethanol is added to provide appropriate binding conditions and the sample is then applied to an RNeasy mini spin column where the total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in 30 µl, or more, of water.

The RNeasy Mini and RNeasy Plant Mini procedures isolate all RNA molecules longer than 200 nucleotides. Small RNAs such as 5.8S RNA, 5S RNA, and tRNAs, approximately 160, 120, and 70–90 nucleotides in length respectively, will not bind quantitatively under the conditions used. Since low-molecular-weight RNA species make up 15–20% of the total RNA, the RNeasy procedure enriches for larger RNA molecules. Therefore, the size distribution of RNA isolated with the RNeasy procedure is comparable to that obtained

by centrifugation through a CsCl cushion, because small RNAs do not sediment efficiently during centrifugation through CsCl.

In this handbook separate protocols are provided for the various starting materials. The protocols differ primarily in the lysis and homogenization of the sample, and in the adjustment of the conditions for binding RNA to the RNeasy membrane. Once the sample is bound to the membrane, the protocols are similar (see flowchart, page 9).

Isolation of Total RNA from Animal Cells and Tissues

Samples (maximum 1 x 10^7 cells or 30 mg tissue, see pages 12–13) are disrupted in lysis buffer containing GITC (Buffer RLT) and homogenized. An overview of disruption and homogenization methods is given on page 16. Ethanol is then added to the lysate, creating conditions which promote selective binding of RNA to the RNeasy membrane. The sample is then applied to the RNeasy mini spin column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water.

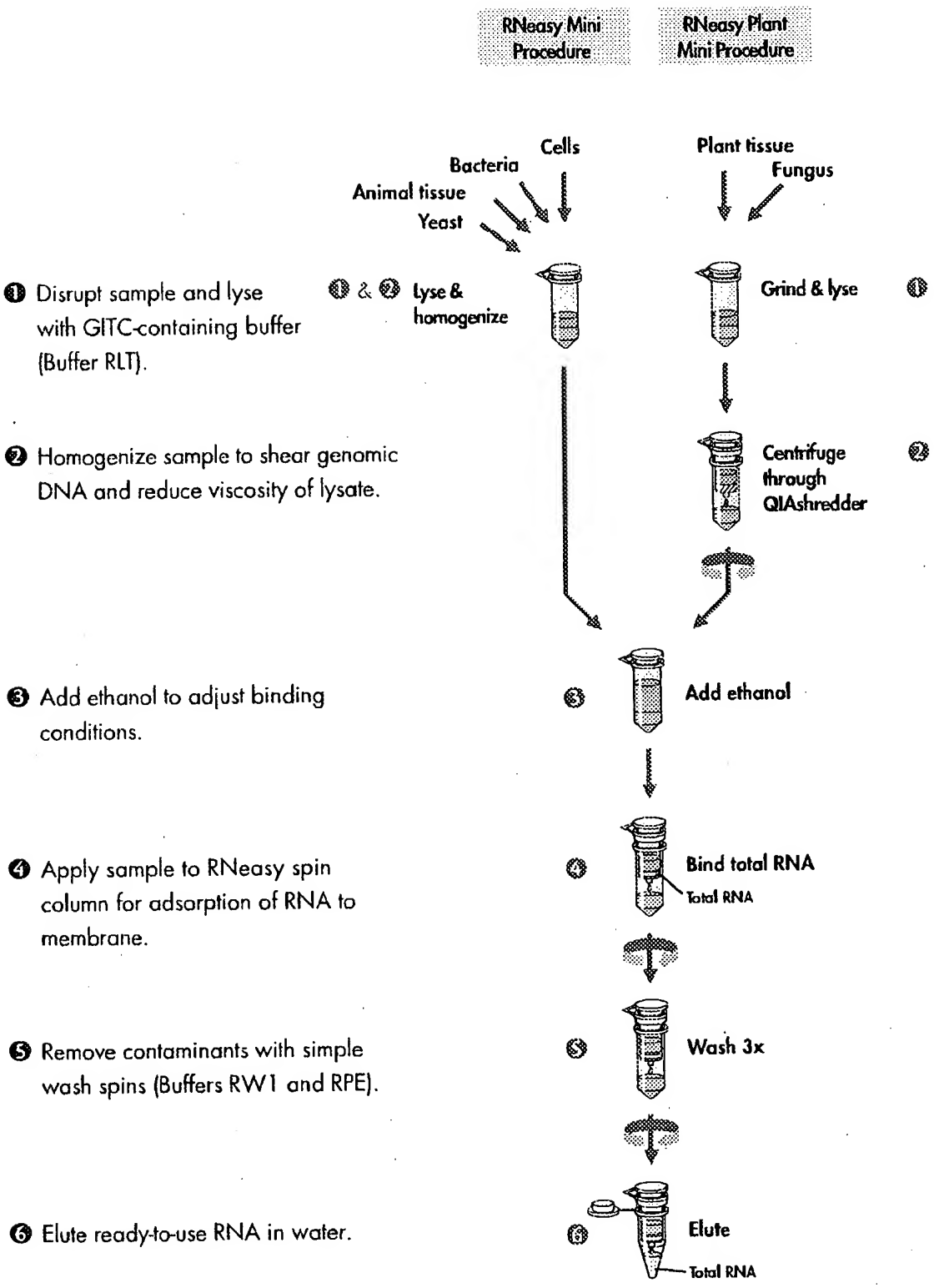
Isolation of Cytoplasmic RNA from Animal Cells

Since the cytoplasm contains RNA in its mature form, this protocol is particularly advantageous in applications where unspliced or partially spliced RNA is not desirable. Cytoplasmic RNA accounts for approximately 85% of total cellular RNA. This protocol is also optimal in applications where the absence of DNA contamination is critical, since the nuclei are removed and no genomic DNA is released.

Cultured cells (maximum 1 x 10^7) are lysed in a buffer (Buffer RLN) containing the non-ionic detergent Nonidet® P-40 which lyses the plasma membrane. Nuclei remain intact during the lysis procedure and are removed by centrifugation. Lysis buffer (Buffer RLT) and ethanol are added to the supernatant to provide optimal conditions for selectively binding RNA to the RNeasy membrane. The sample is then applied to the RNeasy mini spin column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water.

Isolation of Total RNA from Bacteria

Bacteria (maximum 1 x 10^9 cells) are incubated in a buffer containing lysozyme to digest the bacterial cell wall prior to lysis. After addition of GITC-containing lysis buffer (Buffer RLT) and ethanol the sample is loaded onto an RNeasy mini spin column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water.


RNeasy Mini Procedure
RNeasy Plant Mini Procedure
Cells
Bacteria
Animal tissue
Yeast
Plant tissue
Fungus
❶ Disrupt sample and lyse with GITC-containing buffer (Buffer RLT).
❶ & ❷ Lyse & homogenize
Grind & lyse ❶
❷ Homogenize sample to shear genomic DNA and reduce viscosity of lysate.
Centrifuge through QIAshredder ❷
❸ Add ethanol to adjust binding conditions.
❸ Add ethanol
❹ Apply sample to RNeasy spin column for adsorption of RNA to membrane.
❹ Bind total RNA
Total RNA
❺ Remove contaminants with simple wash spins (Buffers RW1 and RPE).
❺ Wash 3x
❻ Elute ready-to-use RNA in water.
❻ Elute
Total RNA

Isolation of Total RNA from Yeast

Two different protocols are provided for the isolation of total RNA from yeast (maximum 5 x 10^7 cells) using RNeasy Mini Kits. The protocols differ primarily in the way the yeast cell walls are disrupted (enzymatically or mechanically). In general, the protocols function equally well. For some applications the Enzymatic Lysis Protocol may be preferable as no additional laboratory equipment is required. However, the Mechanical Disruption Protocol can be used in time-course experiments where enzymatic incubation steps cannot be tolerated.

Enzymatic Lysis Protocol (Standard and Abbreviated Version)

This protocol uses zymolase or lyticase digestion of the cell walls to convert cells to spheroplasts which are processed using the RNeasy Mini Kit. In the standard protocol, spheroplasts are separated from the digestion mixture before lysis by centrifugation. In the abbreviated version of this protocol, for use with up to 2 x 10^7 cells, the digestion mixture is used directly in the RNeasy procedure without prior separation of the spheroplasts. Spheroplasts are lysed in GITC-containing lysis buffer (Buffer RLT) and ethanol is added to the digestion mixture to provide optimal conditions for selectively binding RNA to the RNeasy membrane. The sample is then applied to the RNeasy mini spin column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water.

Mechanical Disruption Protocol

Using this protocol, yeast cells are lysed and homogenized by mechanical disruption during high-speed agitation in a bead mill in the presence of glass beads and GITC-containing lysis buffer (Buffer RLT). Ethanol is added to the lysate creating conditions which promote selective binding of RNA to the RNeasy membrane. The sample is then applied to the RNeasy mini spin column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water.

Isolation of Total RNA from Plant Cells and Tissues and Filamentous Fungi

In the RNeasy Plant and Fungi Protocol, samples (maximum 100 mg) are first ground in liquid nitrogen and then lysed under highly denaturing conditions. The RNeasy Plant Mini Kit includes a choice of lysis buffers, Buffer RLT and Buffer RLC, which contain GITC or guanidine hydrochloride (GuHCl) respectively. The higher cell disruption and denaturing properties of Buffer RLT frequently make it the buffer of choice. However some tissues, such as milky endosperm of maize or mycelia of filamentous fungi, solidify in this buffer making the extraction of RNA impossible. In these cases Buffer RLC should be used. After lysis with either buffer, samples are centrifuged through QIAshredder™, which is supplied with the RNeasy Plant Mini Kit. This simultaneously removes insoluble material and reduces the viscosity of the lysates by disrupting gelatinous material often formed in plant

and fungal lysates. Ethanol is added to the cleared lysate, creating conditions which promote selective binding of RNA to the RNeasy membrane. The sample is then applied to the RNeasy mini spin column. Total RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water.

RNeasy Plant Mini Kits can also be used for total RNA minipreparation from animal cells and tissues, bacteria, and yeast.

RNA Cleanup

RNeasy Mini Kits and RNeasy Plant Mini Kits can be used to purify RNA from enzymatic reactions (e.g. DNase digestion or RNA labeling) or for desalting RNA samples (maximum 100 µg RNA). Lysis buffer and ethanol are added to the sample to provide conditions which promote selective binding of RNA to the RNeasy membrane. The sample is then applied to the RNeasy mini spin column. RNA binds to the membrane, contaminants are efficiently washed away, and high-quality RNA is eluted in water.

Important Points before Using RNeasy Kits

How much starting material can I use?

It is essential to use the correct amount of starting material in order to obtain optimal results. The two main factors used to determine the amount of starting material are:

- The volume of Buffer RLT required for efficient lysis
- The RNA binding capacity of the RNeasy mini spin column (100 µg)

For samples containing very high amounts of RNA, this means that smaller amounts of starting material than listed in Table 1 should be used in order to avoid exceeding the RNA binding capacity of the column. Average RNA yields from various sources are provided in Table 2 and can be used as a guide for calculating amounts of starting material.

For samples containing average or low amounts of RNA, the maximum amount of starting material can be used. In these cases, even though the RNA binding capacity of the column may not be reached, no more starting material may be used or lysis will be incomplete, resulting in lower yields and purity.

Table 1. RNeasy mini spin column specifications

Maximum binding capacity	100 µg RNA
Maximum loading volume	700 µl
RNA size distribution	RNA >200 nucleotides
Minimum elution volume	30 µl
Maximum amount of starting material	
Animal cells	1×10^7*
Animal tissue	30 mg*
Bacteria	1×10^9*
Yeast	5×10^7*
Plant tissue	100 mg
Filamentous fungi	100 mg

* *For larger amounts RNeasy Midi or Maxi Kits are recommended. Please call our Technical Service Group or see ordering information on page 62.*

Note: If the RNA binding capacity of the RNeasy mini spin column is exceeded, yields of total RNA will not be consistent and less than 100 µg of total RNA may be recovered. If lysis of the starting material is incomplete, the yield of total RNA will be lower than expected even if the binding capacity of the RNeasy column is not exceeded.